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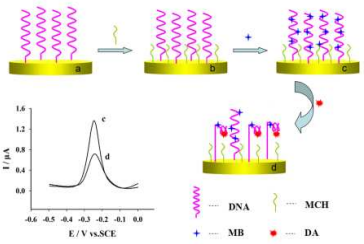


A simple and label-free electrochemical biosensor was developed for sensitive and selective detection of dopamine based on dopamine DNA aptamer

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# A simple label-free electrochemical aptasensor for dopamine detection

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A simple label-free electrochemical biosensor was developed based on dopamine (DA) DNA aptamer immobilized on the Au electrode through the Au-S bond. Taking methylene blue (MB) as the intercalating probe, DA was sensitively and selectively detected. The developed electrochemical DA aptasensor showed a good linear response to DA in the range of 5-150 nmol/L as well as a low detection limit of 1.0 nM (S/N=3). The biosensor also exhibited satisfactory selectivity and could be successfully used to detect DA in human blood, suggesting that the aptasensor has promising application in analysis of DA in real clinical samples.

## Introduction

Dopamine (DA) is a pivotal catecholamine neurotransmitter molecules with a variety of functions in the central nervous system.[1-4] It is reported that DA levels are related to the severity and progression of neurological disorders such as Parkinson's disease, attention-deficit hyperactivity disorder (ADHD) and Huntington's diseases.[5-7] Thus, it is of paramount significance to develop strategies in detecting dopamine precisely and studying the mechanisms of dopamine related diseases. Till now, several methods have been developed in DA analysis, such as high-performance liquid chromatography (HPLC),[8] fluorescence,[9] surface plasma resonance (SPR)[10] and surface-enhanced Raman scattering (SERS).[11] Although these methods were well established, most of them depended on complex sample preparation, expensive instrumental equipments, and are time-consuming.[12,13] Consequently, the developments of simple, sensitive, and cost-effective strategies for DA quantification are highly desired. Due to their low cost, simplicity, rapidity and high sensitivity, electrochemical methods have attracted great attention in quantitative and qualitative assay of analytes.[14-16] Based on the inherent redox activity of DA and excellent characteristics of electrochemical methods, many methods for the direct electrochemical determination of DA have been developed and lots of materials have been used as the active materials to chemically modify the electrode.[17] Among them, the most popularly materials are carbon nanomaterials, such as carbon nanotubes,[18] porous carbon,[19] carbon spheres,[20] graphene,[21] and graphene oxide (GO).[22] However, the analytical performance (such as detection limit, stability and selectivity) of these modified electrodes are not entirely satisfactory to the practical application in biological samples due to the following possible reasons: (1) based on their good electrical conductivity and high surface-to-volume ratios,[23] carbon materials-modified electrodes usually have large background currents which will overlap the oxidation currents of DA in low DA concentration, resulting in the unsatisfactory detection limit. (2) The fouling of the electrode surface by the oxidation products of DA can not be avoided usually, resulting in the poor stability of the sensors.[24] (3) The interference of other neurotransmitter metabolism (NT)[25] can not be avoided easily, leading to the poor selectivity of the related electrochemical DA sensors. Therefore, the sensitive and selective electrochemical assay of DA is still a challenge in real and complex samples.

On the other hand, it is well-known that aptamers are single stranded DNA or RNA molecules selected from random-sequence nucleic acid libraries using systematic evolution of ligands with exponential enrichment (SELEX).[26,27] They can specifically bind to a variety of target molecules with high affinity, such as drugs, proteins, as well as organic and inorganic molecules.[28-30] Due to their distinctive properties, including simple synthesis, easy labeling, good stability, and wide applicability, aptamers have been widely employed as recognition elements for biosensors with electrochemiluminescent (ECL),[31] fluorescent,[32,33] colorimetric,[34,35] electrochemical,[36,37] piezoelectric,[38] and chromatographic[39] methods. Among them, electrochemical aptamer-based (E-AB) biosensors have received great interests because of the simplicity, fast response, relatively cheap cost,

high sensitivity and low power requirement of electrochemical methods. However, only a few works have been reported to electrochemically detect dopamine based on the dopamine aptamer.[40] Farjami[4] developed an E-AB biosensor based on the immobilization of dopamine RNA aptamer on a cysteamine-modified Au electrode to specifically bind dopamine and then the detection of DA was achieved by the direct electrochemical oxidation of DA. As we know, RNA is unstable and hard to be synthesized.[41] On the other hand, the DNA homolog of the RNA aptamer was synthesized and found that the function and specificity of the RNA aptamer were retained.[42] Furthermore, the DNA aptamer of DA has improved affinity relative to the RNA aptamer to bind with the same putative binding site, which indicates that DNA aptamer of DA is superior to the related RNA aptamer. Thus, Zheng[43] developed a colorimetric method to detect DA based on the DNA aptamer of DA as recognition element and unmodified gold nanoparticles as probes. Li[44] designed a multiple-parallel-connected (MPC) silicon nanowire field-effect transistor with DNA-aptamer of DA for sensitive and selective detection of DA. Liu[45] also reported an E-AB biosensor based on the immobilization of DA DNA aptamer on graphene (GR) and polyaniline (PANI) modified electrodes for DA analysis. Although this work showed good analytical performance, the interference of the nonspecific adsorption between dopamine and GR-PANI film may be unavoidable due to the rough surface of the GR-PANI modified electrode. Therefore, further efforts for simple, sensitive, selective determination of DA are still desirable.

In this work, a simple strategy based on DA DNA aptamer as a sensitive and selective bio-recognizer to detect DA was designed. As shown in Figure 1, DNA aptamer was immobilized onto the Au electrode surface through the Au-S bond. 6-Mercapto-1-hexanol (MCH) was used as blocker to prevent nonspecific adsorption from other interferents.[46] The MB molecules were used as signal tag and bound with DNA single strand through electrostatic interaction between DNA phosphate backbones and MB, and specifically interaction between MB and guanine bases.[47] When DA was specifically bound to the DNA aptamer, the MB molecules would be dissociated from the aptamer, resulting in significant decrease of the MB peak current (Fig.1). Based on the change of the MB peak current, DA could be detected sensitively and selectively. This lable-free E-AB biosensor was simple and could have promising application in analysis of DA in real clinical samples.

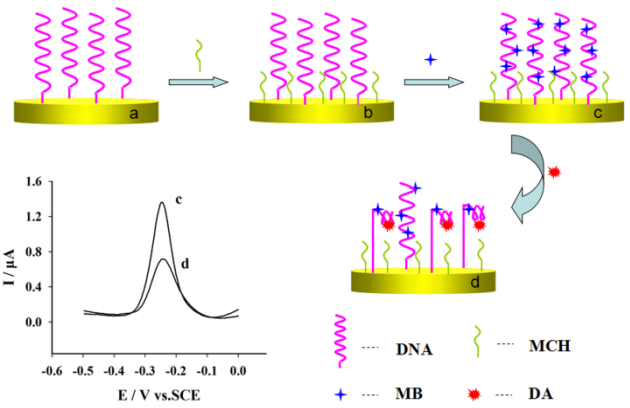


**Fig. 1** Schematic illustration of the lable-free electrochemical biosensor based on DNA aptamer for DA detection.

## Experimental

### Reagents and materials

DNA aptamer was purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). According to the literature,[42-45] the sequence of DNA aptamer is as follows: 5′ - SH - $(CH_2)_6$ - GTC TCT GTG TGC GCC AGA GAA CAC TGG GGC AGA TAT GGG CCA GCA CAG AAT GAG GCC C-3′. $NaH_2PO_4 \cdot 2H_2O$, $K_2HPO_4 \cdot 12H_2O$ and KCl were purchased from Sinopharm Chemical Technology Co.,Ltd. (China). Dopamine(DA), Ascorbic acid (AA), Uric acid (UA), 3,4-dihydroxyphenylacetic acid (DOPAC), Vanillic acid (VA), catechol, Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) and 6-mercaptohexanol (MCH) were purchased from Sigma-Aldrich. Methylene blue (MB) was purchased from Tianjin Hengxing Chemical Reagent manufacturing Co. Ltd. (China). All other chemicals were analytical grade and used without further purification. Aqueous solutions used throughout were prepared with ultrapure water (>18 MΩ cm) obtained from a Millipore system. Human serum was provided by the Affiliated Hospital of Hunan University and stored at 4 °C.

### Preparation of the electrochemical aptasensor

Prior to use, gold electrode was polished to a mirror-like surface with 0.5 and 0.05 μm alumina slurries, followed by ultrasonication in ultrapure water and anhydrous ethanol. The electrode was then pretreated electrochemically in 0.5M $H_2SO_4$ aqueous solution by potential cycling in the potential range of −0.3 to 1.6 V at a scan rate of 100 $mVs^{-1}$ until the cyclic voltammogram characteristic for clean gold electrode was obtained. Then, the gold electrode was washed thoroughly with copious amount of ultrapure water and dried under nitrogen gas.

For the preparation of the aptamer modified electrode, 5 μL of a 10 μM DNA aptamer solution in 0.2 M PBS (pH 7.0) was dropped onto the cleaned gold electrode surface for 16 h at



 

room temperature to obtain the DNA/Au electrode. The DNA/Au electrode was further immersed in 1 mM MCH solution with 0.2 M PBS for 1 h to block the uncovered gold electrode surface as well as to make the array of ss-DNA on the electrode interface more regularly, and the obtained electrode was labeled as MCH/DNA/Au electrode. To monitor each immobilization step, the electrochemical impedance measurements were performed in 0.1 M KCl aqueous solution with 5 mM $[Fe(CN)_6]^{3-/4-}$ as the probe.

### Electrochemical measurements

In order to detect DA, the MCH/DNA/Au electrode was firstly immersed into 20 mM MB solution for 30 min to adsorb MB molecules and the obtained electrode was named as MB/MCH/DNA/Au electrode. Then, the MB/MCH/DNA/Au was incubated in 0.2 M PBS (pH 7.0) without or with different concentration of DA solutions for 1h and the peak current of MB was monitored by electrochemical square-wave voltammetry (SWV).

All electrochemical measurements were performed on a CHI 660B Electrochemical Workstation (Chenhua Instrument Company of Shanghai, China). A conventional three-electrode cell was used with a planar gold electrode (2mm in diameter) as the working electrode, a platinum wire as the counter electrode, and a saturated calomel electrode (SCE) as the reference electrode.

## Results and discussion

### Electrochemical characterization of the DNA-modified electrodes

Fig. 2 shows the electrochemical impedance results of the bare Au (a), DNA /Au (b), MCH/DNA/Au (c) electrodes. In the Nyquist plots of impedance spectra, a linear portion at the lower frequencies is attributed to a diffusion-limited process, while the semicircle portion of the plot at the higher frequencies corresponds to the charge transfer process. The increase of the diameter of the semicircle reflects the increase of the interfacial charge-transfer resistance ($R_{ct}$). It is noted that the bare Au electrode shows a very small semicircle domain ($R_{et}$=100 Ω, Fig. 2, curve a), indicating a very fast electron-transfer process of $[Fe(CN)_6]^{3-/4-}$.[22] The self-assembly layer of the negatively charged DA aptamer on the Au electrode surface effectively repels the $[Fe(CN)_6]^{3-/4-}$ anions and thus leads to a significantly enhanced charge-transfer resistance ($R_{et}$=19000 Ω, Fig. 2, curve b). This implies that DA aptamer is successfully immobilized on the surface of Au electrode. When the DNA/Au electrode is blocked by MCH, the value of $R_{et}$ is further increases to 33000 Ω (Fig.2, curve c). All these experimental results indicate that all the steps shown in Fig. 1 were successfully performed.

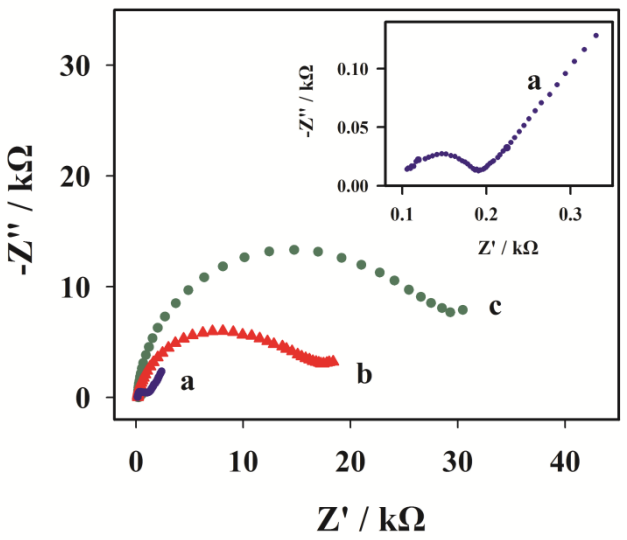


**Fig. 2** Electrochemical impedance spectra (Nyquist plots) of the bare Au (a), DNA/Au (b), MCH/DNA/Au (c) electrodes in 0.1 M KCl aqueous solution containing 5 mM (1:1) $[Fe(CN)_6]^{3-/4-}$ as the redox probe.

### Feasibility of the DA detection at the MCH/DNA/Au electrode

In order to evaluate the feasibility of the DA detection based on the MCH/DNA/Au electrodes, the electrochemical performance of the MCH/DNA/Au and MB/MCH/DNA/Au electrode before and after incubation with 100 nM DA was investigated by SWV and the corresponding results are shown in Fig. 3(A). From Fig. 3(A), It is noted that a remarkable peak current can be observed at -0.25 V on the MB/MCH/DNA/Au electrode (Fig. 3(A), curve a), while no obvious peak current appears on the MCH/DNA/Au electrode (Fig. 3(A), curve c). This suggests that the peak current of MB should result from the MB molecules bound in the DNA strands (DA aptamer). On the other hand, after the MB/MCH/DNA/Au electrode is treated with 100 nM DA solution, the peak current of MB decreases from 1.36 to 0.70 μA (Fig. 3(A), curve b). Furthermore, a control experiment demonstrates that the peak current of the MB/MCH/DNA/Au electrode in 0.2 m PBS (pH 7.0) without DA is relatively stable after 10 min, although an obvious decrease of the peak current is observed in 10 min (Fig. 3B).[46] These results indicate that the above decrease of the peak current of MB molecules should result from the conformation change of DA aptamer due to the specific recognition of DA aptamer to DA molecule. The results from Fig. 3 imply that the MB/MCH/DNA/Au electrode may be used to detect the concentration of DA.

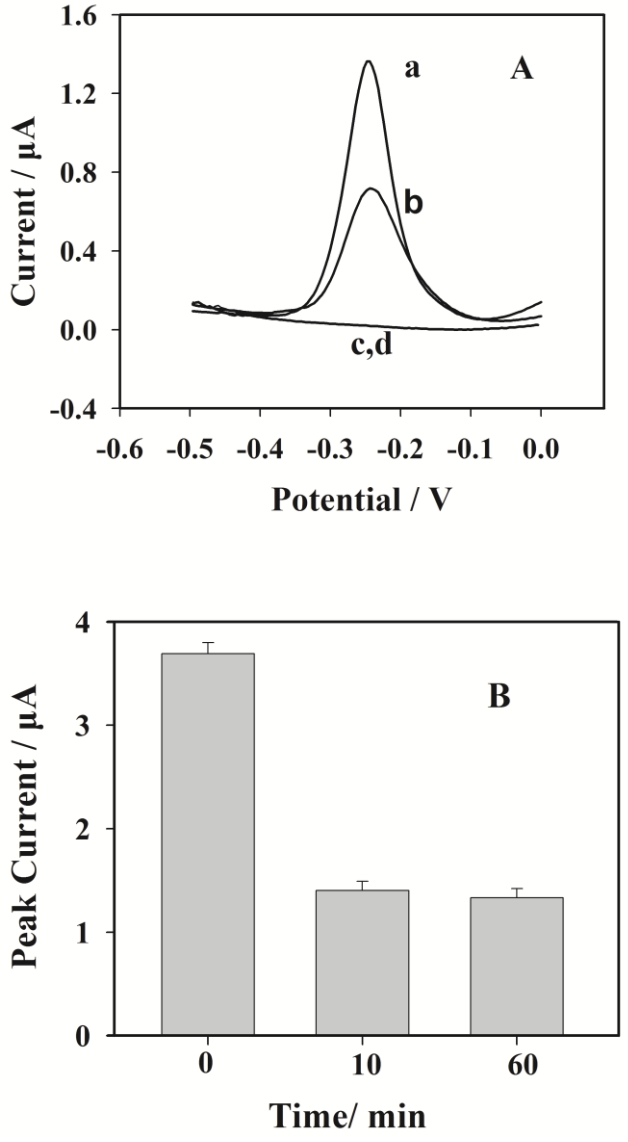


**Fig. 3 (A)** SWV curves of the MB/MCH/DNA/Au (a, b) and MCH/DNA/Au (c, d) electrodes before (a, c) and after (b, d) incubation with 100 nM DA in 0.2 M PBS (pH 7.0). **(B)** The peak current of MB obtained by immersing MB/MCH/DNA/Au electrodes in MB-free PBS( 0.2M, pH 7.0) with different time.

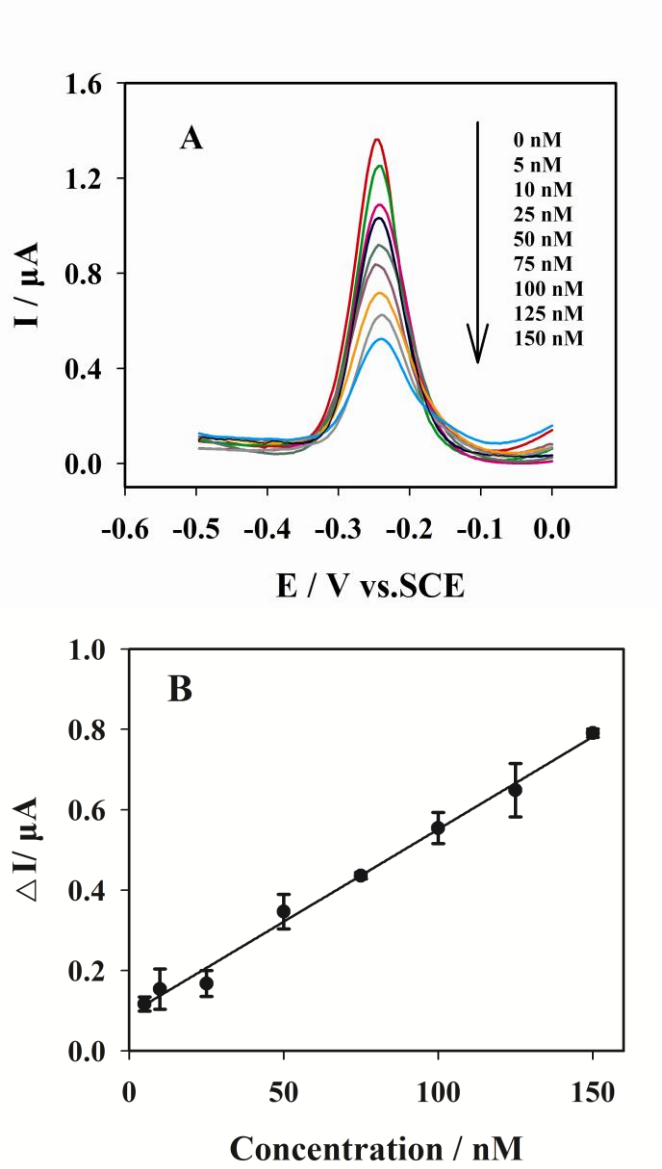


**Fig. 4** (A) SWV curves of MB in 0.2 M PBS (pH 7.0) obtained on the MCH/DNA/Au electrode after treatment in DA solutions with different concentration. (B) The linear fit plot of current difference (ΔI) as a function of the DA concentration. $\Delta I = I_0 - I$, $I_0$ and I are the peak currents of MB obtained on the MB/MCH/DNA/Au electrodes before and after treated with different concentration of DA, respectively.

**Electrochemical detection of DA**

Fig. 4A shows the SWV responses of MB/MCH/DNA/Au electrode after treated with different concentrations of DA. It is noted clearly that the peak current of MB decreases with the increase of the concentration of DA. Fig. 4(B) shows more clearly the relationship between the peak current of MB and the concentration of DA. It is noted that the calibration plot exhibits a linear range from 5 nM to 150 nM and the detection limit is 1.0 nM (S/N= 3) .The corresponding linear equation is ΔI (μA)=0.0912+0.0046c (nmol/L) (R=0.9968).

Furthermore, the analytical performance obtained at the MB/MCH/DNA/Au electrode was compared with those reported in the literatures (Table 1). From Table 1, it is noted that the detection limit obtained by this method is much lower than that by common chemically modified electrodes (such as carbon nanomaterials-modified electrodes),[18-22] DA RNA aptamer-based electrochemical biosensor[4] and DA DNA aptamer-based colorimetric method.[43] On the other hand, the preparation procedure of the MB/MCH/DNA/Au electrode is very simple, which may result in excellent reliability in the daily determination of DA in real clinical samples.



 

**Table 1** Comparisons of the different modified electrodes for the electrochemical determination DA

| Electrode materials | Method | Detection limit [μM] | Linear range [μM] | Ref. |
|---|---|---|---|---|
| carbon nanotubes | DPV[b] | 0.8 | 3–200 | 18 |
| porous carbon | DPV | 0.011 | 0.5–30 | 19 |
| carbon spheres | DPV | 0.012 | 0.5–90 | 20 |
| graphene | DPV | 0.1 | 2-2500 | 21 |
| graphene oxide | Amperometric | 0.039 | 0.1-175 | 22 |
| RNA DA aptamer | CV[c] | - | 0.1-5 | 4 |
| DNA DA aptamer | Colorimetry | 0.36 | 0.54-5.4 | 43 |
| DNA DA aptamer | SWV | 0.001 | 0.005-0.15 | This work |

**Selectivity, reproducibility and stability**

Under physiological conditions, the co-existence of other NTs is a big challenge for the specific evaluation of DA in brain. Therefore, taking ascorbic acid(AA), uric acid(UA), 3,4-dihydroxyphenylacetic acid (DOPAC), vanillic acid (VA) and catechol as the interferents, the selectivity of the developed DA aptasensor has been investigated. As shown in Fig. 5, the response signals caused by these interferents are very small, while the DA sample gives obvious change of the electrochemical signal. These indicate that the proposed biosensing system has an excellent selectivity for DA assay.

The reproducibility of the developed method was also evaluated. Five modified electrodes were used to detect DA (100 nM) and the relative standard deviation is 4.1%. Furthermore, the MB/MCH/DNA/Au electrode remains bioactive after two-week storage at 4 ˚C and the response currents have no significant changes. These demonstrate that the developed E-AB biosensor has satisfactory reproducibility and stability.

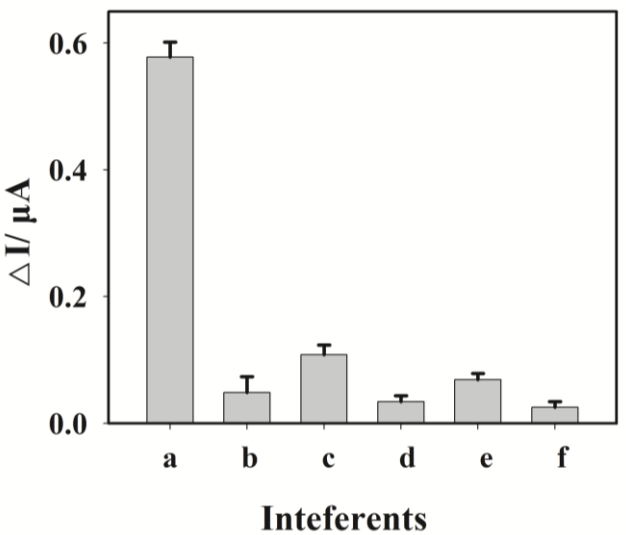


**Fig. 5** The selectivity of the MB/MCH/DNA/Au electrode. $\Delta I = I_0 - I$, $I_0$ and I are the peak currents of MB obtained on the MB/MCH/DNA/Au electrodes before and after treated with the following targets : DA (100 nM ) (a), AA (1 μM ) (b), UA (1 μM ) (c), DOPAC (1 μM ) (d), VA (1 μM ) (e), catechol (1 μM ) (f).

### Recovery test

The recovery experiments for different DA concentrations were carried out by the standard addition method to evaluate applicability and reliability of the developed E-AB biosensor in complex system. The blood serum samples (one part of the blood serum was diluted in ten parts of 0.2 M PBS (pH 7.0)) were employed in this work as the model of the complex systems. As shown in Table 2, the recoveries for the added DA with 10 nM, 50 nM, 100 nM are 99.6 %, 105.5 %, 106.1 %, respectively. These results reveal that the recovery of the developed aptasensor is satisfactory and has great practical applications in DA assay.

**Table 2** Determinations of DA in human serum samples.

| Samples | Added (nM) | Found(nM) | Recoverry(%) |
|---|---|---|---|
| 1 | 10 | 9.96 | 99.6 |
| 2 | 50 | 52.77 | 105.5 |
| 3 | 100 | 106.1 | 106.1 |

## Conclusions

Taking the peak current of MB as signal, a simple and lable-free E-AB biosensor for DA detection was developed. It has excellent analytical performance: the linear range is 5 nM to 150 nM and the detection limit is 1.0 nM (S/N = 3). The biosensor also exhibited excellent selectivity and could be successfully used to detect DA in human blood. Compared with the previously reported methods, the developed method has much lower detection limit than the common chemically modified electrodes (such as carbon nanomaterials-modified electrodes), DA RNA aptamer-based electrochemical biosensor and DA DNA aptamer-based colorimetric method. These suggest that the proposed aptasensor has promising application in analysis of DA in real clinical samples.

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## Notes and references

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[b]Differential pulse voltammetry (DPV), [c]Cyclic voltammetry (CV), [d]Square wave voltammetry (SWV).

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